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(54) Immunoassay for h. pylori in fecal specimens

(57) An immunoassay is described for *H. pylori* in fecal specimens. A fecal specimen suspected of carrying *H. pylori is* dispersed in a sample diluent. The fecal specimen in the diluent is contacted with a first polyclonal antibody for *H. pylori* antigen to form a complex of the antibody and the antigen. The specimen and complex are separated. The complex is exposed to a second polyclonal antibody for the antigen and a portion of the antibody reacting with the complex. Either the first or second antibody is bound to a solid carrier and the other is labelled with a detection agent. The amount of the labelled antibody is determined and from this the presence or otherwise of *H. pylori* antigen in the fecal specimen is determined.

EP 0 806 667 A1

Description

This invention relates to a method for detecting *Helicobacter pylori* in fecal specimens.

H. pylori is a bacterium that is found in the upper gastrointestinal tract of humans which has been implicated in gastroduodenal diseases such as peptic ulcers, gastritis and other maladies. The bacterium was originally classified as a Campylobacter and then reclassified as a Heliobacter based on more detailed information regarding its ultrastructure and fatty acid composition.

A number of different techniques, both invasive and noninvasive, have been used to detect *H. pylori*. The invasive techniques involve gastric biopsies and cultures. The noninvasive techniques include a urea breath test, in which the patient is given C-13 or C-14 labelled urea with a beverage, and the detection of *H. pylori* antibody in sera using antigens in enzyme-linked immunosorbent assays (ELISA). Examples of the latter techniques are found in U.S. Patent 5,262,156 to Aleonohammad and European Patent Application 0 329 570 to Blaser.

Several major antigens have been identified and used in immunoassays in the detection of *H. pylori* antibodies. However, these assays have not exhibited the specificity and sensitivity that are desired in serodiagnosis. Newell, D.G., et al. *Serodian. Immunother. Infec. Dis.,,* 3:1-6 (1989). One problem with these immunoassays is cross-reactivity. Studies of the dominant antigens in *H. pylori*, in particular, the putative flagellar protein, which has a molecular weight of 60 Da, have shown that some of these antigen are not specific to *H. pylori* and also found in other bacteria such as *C. jeuni* and *C. coli*. A second problem that has been encountered in designing immunoassays for *H. pylori* is strain variation. Substantial differences in the antigens has been observed in different strains of *H. pylori*. These problems preclude designing an assay around the use of a single antigen. They also rule out the use of monoclonal antibodies. One approach that has been taken to improving the specificity and selectivity of antibody immunoassays for *H. pylori* has been to use a mixture of antigens from different *H. pylori* strains which mixture is enriched with certain antigen fragments. One ELISA which detects *H. pylori* antibodies in a blood sera is commercially available from Meridian Diagnostics. This assay uses a bacterial whole cell lysate as the antigen.

There are certain disadvantages to using an ELISA which employs antigens to detect the presence of *H. pylori* antibodies. In particular, the antibody titer in human sera remains high for a prolonged time (in some cases as much as six months) after the infection has been treated. Consequently, a positive test using this ELISA does not necessarily mean that the patient is currently infected and requires treatment for *H. pylori* infection. When confronted with a positive ELISA, treating physicians often order a gastric biopsy to confirm the presence of the bacteria before initiating antibiotic therapy. Therefor, the antigen-based ELISA does not eliminate the need for the invasive procedure. By contrast, if an immunoassay could be designed for detecting *H. pylori* antigen instead of the antibody, the need to obtain gastric biopsies to confirm infection could be reduced significantly because the antigen generally can not be detected in a patient within days of its treatment. Thus, there is a need for an ELISA which detects *H.pylori* antigen and, more particularly, there is a need for an ELISA for detecting *H. pylori* directly from fecal specimens.

While ELISA's for detecting microorganisms such as *C. difficile* and adenovirus in fecal specimens are known, in studies of patients with gastric biopsies which are positive for *H. pylori*, the bacteria ordinarily can not be cultured and isolated from the fecal specimens. This and the problems of cross reactivity and strain variation raised serious doubts that an ELISA could be designed that would be specific for *H. pylori* and sensitive enough to reliably detect *H. pylori* antigen directly from a fecal specimen.

In a first aspect thereof, the present invention provides a method for detecting *H. pylori* in fecal specimens which comprises:

(a) dispersing a fecal specimen suspected of carrying *H. pylori* in a sample diluent;
(b) contacting the fecal specimen in the diluent with a first polyclonal antibody for *H. pylori* antigen to form a complex of the antibody and the antigen;
(c) separating said specimen from said complex;
(d) exposing the complex to a second polyclonal antibody for said antigen and a portion of the antibody reacting with said complex, one of said first and second antibody being bound to a solid carrier and the other being labelled with a detection agent; and
(e) determining the amount of the labelled antibody and in turn determining the presence of *H. pylori* antigen in said fecal specimen.

In a preferred embodiment, the first antibody is bound to a carrier and the second is labelled with an enzyme. Triple sandwich assays are also provided.

The invention provides, in a second and alternative aspect thereof, a process for the determination of *H. pylori* in a fecal specimen which comprises:

(a) dispersing a fecal specimen suspected of carrying *H. pylori* in a sample diluent;
(b) contacting the fecal specimen in the diluent with a first polyclonal antibody for *H. pylori* antigen bound to a solid carrier and a second labelled polyclonal antibody for *H. pylori* to form a complex of the antibodies and the antigen;
(c) separating said specimen and said complex;
(d) determining the amount of the labelled antibody and in turn determining the presence of *H. pylori* antigen in said fecal specimen.

According to the third alternative aspect of this invention, there is provided a process for the determination of *H. pylori* in a fecal specimen which comprises:

(a) dispersing a fecal specimen suspected of carrying *H. pylori* in a sample diluent:
(b) contacting the fecal specimen in the diluent with a first polyclonal antibody for *H. pylori* antigen produced by a first antibody-producing species and bound to a solid carrier to form a complex of the antibody and the antigen;
(c) separating said specimen and said complex:
(d) contacting the antibody-antigen complex formed in step (b) with a primary polyclonal antibody for *H. pylori* antigen obtained from a second antibody-producing species to produce a antibody-antigen-antibody complex;
(e) removing the primary antibody not present in the complex from step (d);
(f) contacting the antibody-antigen-antibody complex formed in step (d) with a secondary antibody, said secondary antibody being an antibody for the second antibody-producing species, whereby said secondary antibody forms a complex with said antibody-antigen-antibody complex: and
(g) determining the presence of *H. pylori* antigen in said fecal specimen.

Accordingly, there is provided, in a fourth alternative aspect of this invention, a kit for the determination of *H. pylori* in a fecal specimen including a plate of wells having bound thereto polyclonal antibody for *H. pylori* antigen, a protein-based sample diluent, a conjugate of an enzyme polyclonal antibody for *H. pylori* antigen, wash buffer and, a substrate solution.

Our immunoassay employs polyclonal antibodies for *H. pylori*. These antibodies can be obtained from the sera of a sensitized animal. Sensitization can be accomplished by injecting the antigen into an antibody producing species, typically a mammal and preferably a rabbit, goat or cow. Usually an initial injection is given followed by subsequent booster injections to maximize the response.
Optimally, the injection regime is in multiple doses given to White New Zealand rabbits. The amount of antigen injected must be adequate to elicit a sufficient amount of antibody to be detectable. Antibody production is verified using a trial bleed and Indirect Fluorescent Assay.

H. pylori cells from ATCC strain 43504 have been found to be particularly useful in producing the polyclonal antibody. As previously mentioned, substantial strain variation has been observed in *H. pylori*. Differences in the organism have been observed in different geographic regions as well as dietary groups. However, antibodies obtained through sensitization using cells from strain 43504 have been found to be useful in det cting the organism across geographic regions and dietary groups If necessary, for example, if it is found that the ELISA is not effective in detecting the organism in certain populations, cells from more than one strain of H. pylori could be used to produce the antibody.

The same labels used in known immunometric assays can be used to label our polyclonal antibody

Among these may be mentioned fluorogenic labels for detection by fluorimetry as described in U.S. Patent No. 3,940,475, enzymatic markers as described in U.S. Patent No. 3,654,090, and radioisotopes such as Iodine-125. One of the most common enzymatic markers is horseradish peroxidase (HRP) and alkaline phosphatase enzyme. Example 3 below illustrates labeling polyclonal antibodies with HRP.

The unlabeled polyclonal antibody used in our process to extract the antigenic substance from the fecal specimen being tested can be immobilized on any of the supports commonly used in immunometric assays. Among those that may be used are filter paper, plastic beads, polyethylene, polystyrene, polypropylene or other suitable test tube. The techniques for bonding antibodies to such materials are well known to those skilled in the art.

To prepare the fecal specimen for use in the assay, the specimen is dispersed in a protein-based sample diluent. The diluent be formulated and buffered to minimize cross-reactivity. As examples of sample diluents, mention can be made of fetal bovine serum, normal goat serum, guinea pig serum, horse serum, casein, albumin, gelatin, and bovine serum albumin (BSA). A dilution of one part fecal specimen and four parts diluent has been found to be useful. In addition to using the protein based additives, cross-reactivity can be reduced by the addition of detergents and increasing or decreasing pH or ionic strength of the diluent buffer. For example, many sample diluents contain Triton X-100 and/or Tween 20 at concentrations ranging between 0.05% and 2%. NaCl can be added in the ranges between 0-2.9% to alter the ionic strength of the buffer system. These changes lead to greater specificity by reducing the likelihood of weak or non-specific interactions from forming.

Cross-reactivity can also be addressed in the formulation of the antibody solutions and the washes that are used in the assay. The antibody can be provided in a buffered solution in conjunction with one of the protein sera mentioned previously. The washes used in the assay can be formulated and buffered by the addition of salts and surfactants to control cross-reactivity. A preferred wash for reducing cross-reactivity is a phosphate buffered saline solution.

The preparation of the antigen, production of the polyclonal antibodies and an ELISA are illustrated in more detail by the following non-limiting examples.

Exampl 1

Pr paration of th *Helicobacter pylori* (*H. pylori*) antigen:

H. pylori (ATCC strain 43504) was streaked for isolation on Tryptic Soy Agar (TSA) supplemented with 5% defibrinated sheep blood. The plate was incuba[illegible] 7°C in a microaerophilic environment for [illegible] [illegible]ultant bacterial growth was evaluated by use of colony morphology. urease. catalase and oxidase reactions, and gram stain Acceptable growth was subcultured to four TSA with sheep blood agar plates and grown at 3[illegible] in a mi[illegible]ophilic environment for 3-4 days.

Each plate was flo[illegible] of 0.85% NaCl and the bacterial grow[illegible] a plate spreader. The bacteria were centrifuged [illegible]00xg for 15 minutes at 2-8°C. Each pellet was [illegible]nded in 3ml of 0.85% NaCl and combined to one [illegible]uge container. The bacterial suspension was centrifuged at 10,000xg for 15 minutes at 2-8°C. The pellet was resuspended and centrifuged as before. The final pellet was resuspended to 3% of the total original volume in 20mM phosphate buffer. The bacterial cells were transferred to an iced container and sonicated 5 times for 3 minutes at the maximum setting that does not cause foaming, resting for 30 seconds in between cycles. The sonicated bacterial cells were centrifuged at 57,000xg for 15 minutes at 2-8°C. The bacterial supernatant was collected and the pellet discarded.

Example 2

Production of Rabbit Polyclonal:

The bacterial supernatant obtained in Example 1 was diluted in equal parts with Freunds complete adjuvant (total immunogen is 1.0ml) to provide 1 x 10^8 cells per ml. This solution was mixed thoroughly and 0.2-0.5ml of the solution was injected intramuscularly into the right hind leg and 0.1-0.25ml of the solution was injected subcutaneously into each of eight to ten sites on the back. Subsequent injections were one month apart using Freunds incomplete adjuvant and the injection sites were limited to subcutaneous back.

A trial bleed was taken after three months. The bleed was taken from the central ear vein one week after the third injection. This bleed was incubated overnight at 2-8°C. The next day the blood was centrifuged at 5,000xg for 15 minutes at room temperature. The supernatant was collected and the pellet discarded. The supernatant was tested by an Indirect Fluorescent Assay (IFA). The IFA was performed by placing 10ul of *H. pylori* suspension on glass slides and heat fixed. The slides were blocked with 3% bovin serum albumin (BSA) for 5 minutes then washed with a wash of phosphate buffered saline (PBS) and 0.5% Tween 20 (PBS/Tween wash). 50µl of the trial bleeds and normal rabbit serum, as a control, diluted 1:10 in phosphate buffered saline with sodium azide (PBSA) were added and incubated in a humid environment for 30 minutes. After washing with PBS/Tween wash, Goat anti-Rabbit conjugated to FITC (fluoroescein isothiocyanate) was diluted 1:10 in PBSA and 50µl was added to each well. The slides were incubated for 30 minutes in a dark humid [illegible] The slides were washed again. Fluores[illegible]unting media and a cover slip were add[illegible] a fluorescence microscope. Rabbits whose [illegible]strated a 4+ fluorescence intensity reading [illegible]lume bled.

The vo[illegible] was obtained similarly to the trial bleed except 50[illegible] was removed from each rabbit. The blood was incubated and centrifuged as the trial bleed was.

The total volume of sera was determined and an equal volume of phosphate buffer saline (PBS) was added. A 40% ammonium sulfate precipitation was performed to remove unnecessary protein and incubated at 2-8°C for 24 hours. The mixture was transferred to a centrifuge tube and centrifuged at 10,000xg for 30 minutes at room temperature. Resuspend the pellet in PBS to approximately one third of the original volume. The suspension was dialyzed against 200 times the total suspension volume of 0.0175M potassium phosphate, pH 6.5 at 2-8°C. After the dialysis, the suspension was centrifuged at 10,000xg for 20 minutes at room temperature. The supernatant was collected and the pellet was discarded.

A DEAE (diethylaminoethyl cellulose) column was equilibrated with 0.0175M potassium phosphate, pH 6.5 at room temperature. The supernatant was placed over the column and the effluent fractions collected. A protein concentration (OD_{280}) was determined and all fractions greater than 0.200 were pooled. The pooled antibody was tested in the ELISA.

Example 3

Horseradish peroxidase conjugation:

The conjugation used 10mg of DEAE purified rabbit anti-*H. pylori* antibody. The antibody was brought to a final volume of 2.5ml by concentration or by the addition of 10mM sodium bicarbonate pH 9.6. A PD-10 column (Pharmacia) was equilibrated with 10mM sodium bicarbonate pH 9.6. The antibody was added to the column and nine fractions of 1.0ml were taken. A protein concentration (OD_{280}E.O.=1.4) of each fraction was taken and those reading above 0.200 were pooled.

A separate PD-10 column was equilibrated with 1mM sodium acetate trihydrate pH 4.3. The minimum amount of Horseradish Peroxidase (HRP) used was 1.172mg HRP for each lmg of antibody. 1-1/2 times the calculated minimum HRP was weighed out and added to 1.0ml of deionized water. A protein concentration

(OD_{403}E.O.=2.275) was performed and HRP diluted to 10mg/ml with deionized water. 0.1M sodium m-periodate was added at a concentration of 0.2ml for every 4mg HRP. This reaction was allowed to proceed for 20 minutes at room temperature with gentle rocking. The reaction was stopped by the addition of 50 μl of 2M ethylene glycol for every ml of HRP at 4mg. The HRP was eluted through the PD-10 with 1mM sodium acetate trihydrate pH 4.3.

The conjugation ratio was lmg antibody to 1.172mg HRP. The antibody was adjusted with 10mM sodium bicarbonate pH 9.6 and the HRP with 1mM sodium acetate trihydrate pH 4.3. The two were combined in a dedicated flask and pH adjusted with 0.2M sodium bicarbonate, pH 9.6 to 9.6. Protected from light, the mixture was incubated for two hours on a rotator at 85-95rpm at room temperature. After two hours, 0.1ml of 4mg/ml of sodium borohydride was added for every 8mg of antibody. The new mixture was incubated at 4°C for two hours on a rotator. The conjugate was passed through a PD-10, equilibrated with PBS, and fractions containing the conjugate were collected. The fractions were pooled and concentrated to approximately 1.0ml. The concentrate was placed over a Sephracryl S-200 column equilibrated with PBS at a flow rate of 10ml/hr. 2.0ml fractions were collected and a protein concentration of both the antibody and the HRP were performed. The fractions with a simultaneous peak in both the OD_{280} and OD_{403} were pooled and concentrated to approximately 1.0mg/ml.

Example 4 below illustrates a so called "forward" assay in which the antibody bound to the support is first contacted with the specimen being tested to extract the antigen from the sample by formation of an antibody/antigen complex and contacting the complex with a known quantity of labelled antibodies. However, those skilled in the art will appreciate that the immunometric assay can also be conducted as a so called "simultaneous" or "reverse" assay. A simultaneous assay involves a single incubation step as the antibody bound to the solid support and the labelled antibody are both added to the sample being tested at the same time. After the incubation is complete, the solid support is washed to remove the residual sample and uncomplexed labeled antibody. The presence of the labeled antibody associated with the solid support is then determined. A reverse assay involves the step wise addition first of a solution of labeled antibody to the fecal specimen followed by the addition of the unlabeled antibody bound to the support. After a second incubation, the support is washed in a conventional fashion to free it of the residual specimen and the unreacted labelled antibody.

Example 4

ELISA Test:

The antibody was diluted in PBS serially between 20μg/ml and 2.5μg/ml. A 0.100ml aliquot of each dilution was added to an Immunlon-II strip (Dynatech), covered and incubated overnight at room temperature. The plate was washed once with PBS/Tween wash. It was blocked with 1%BSA/PBS for 1 hour at room temperature. Again washed once with PBS/Tween wash. Several positive and negative samples were diluted 1:5 in 0.1%BSA/PBS. Each sample (0.100ml) was added to a well of the strips, covered and incubated 1 hour at room temperature. The plate was then washed 5 times with Merifluor C/G wash. A previously accepted rabbit anti-*H. pylori* conjugated to horseradish peroxidase was diluted to 10μg/ml and 0.100ml added to each well. The plate was covered and incubated at room temperature for 1 hour. Again washed 5 times with PBS/Tween wash then developed for 10 minutes at room temperature with 0.100ml of trimethylbensidine (TMB) solution. Stopped with 0.050ml $2NH_2SO_4$ and read after two minutes. The dilution yielding the maximum signal and lowest background was chosen as the optimal dilution.

Quantitative determinations can be made by comparing the measure of labeled antibody with that obtained for calibrating samples containing known quantities of antigen. Table 1 below shows the optical density obtained when four samples, each containing a predetermined number of organisms, is run through the assay.

Table 1

No. of organisms per ml	$OD_{450/630}$
3 X 10^7	2.547
1.9 X 10^4	0.662
4.6 X 10^6	0.182
1.1 X 10^6	0.038

The results of running six clinical specimens through the assay are shown in Table 2.

Sample	$OD_{450/630}$	Result
1	0.301	Positive
2	0.713	Positive
3	0.284	Positive
4	0.005	Negative
5	0.033	Negative
6	0.008	Negative

So-called triple sandwich assays can also be used for detecting *H. pylori* in fecal specimens in accordance with the invention. Triple assays are know in the art and the basic methodology can be applied to the detection of *H. pylori* in fecal specimens. A triple assay is typically conducted by dispersing a fecal specimen suspected of carrying *H. pylori* in a sample diluent which minimizes cross-reactivity and adding the diluted sample to an immobilized antibody for *H. pylori* that has been obtained from a first species of an antibody producing animal. The

sample is incubated to form the antibody-antigen complex. After washing excess specimen from the immobilized support, an *H. pylori* antibody known as a primary antibody and obtained from a second species of an antibody producing animal is added to the antibody-antigen complex and incubated to form an antibody-antigen-antibody complex. After forming this complex and removing the unreacted antibody, the complex is reacted with an antibody known as a secondary antibody which is an antibody to the second antibody producing species such as anti-(rabbit, cow or goat) immunoglobulin. The secondary antibody is labelled in a conventional manner, typically with an enzyme, and incubated with the antibody-antigen-antibody complex to form a triple antibody complex or sandwich. After removing the unreacted secondary antibody, the antigen is assayed in a conventional manner. In using an enzyme label, a substrate is added to the complex of the antigen and the three antibodies and the reaction of the substrate with the linked enzyme is monitored to determine the amount of the antigen present in the specimen. In the triple sandwich assay, as in the basic sandwich assay, the washes and the antibody solutions are formulated or buffered to control cross-reactivity as needed.

Claims

1. A process for the determination of *H. pylori* in a fecal specimen which comprises:

   (a) dispersing a fecal specimen suspected of carrying *H. pylori* in a sample diluent;
   (b) contacting the fecal specimen in the diluent with a first polyclonal antibody for *H. pylori* antigen to form a complex of the antibody and the antigen;
   (c) separating said specimen and said complex;
   (d) exposing the complex to a second polyclonal antibody for said antigen and a portion of the antibody reacting with said complex, one of said first and second antibody being bound to a solid carrier and the other being labelled with a detection agent; and
   (e) determining the amount of the labelled antibody and in turn determining the presence of *H. pylori* antigen in said fecal specimen.

2. A process according to Claim 1, wherein the first antibody is bound to a solid carrier and the second antibody is labelled with a detection agent.

3. A process according to Claim 1, wherein the first antibody is labelled with a detection agent preferably selected from alkaline phosphatase and beta galactosidase horseradish peroxidase, and the second is bound to a solid carrier.

4. A process according to any preceding claim, wherein the sample diluent is a protein based diluent, preferably a diluent containing a protein selected from fetal bovine serum, normal goat serum, guinea pig serum, horse serum, casein, albumin, gelatin, and bovine serum albumin.

5. A process according to any preceding claim, wherein said polyclonal antibody is obtained by sensitizing an antibody-producing mammal with *H. pylori* cells.

6. A process according to Claim 5, wherein the cells are cells from a plurality of *H. pylori* strains.

7. A process according to Claim 5, wherein said cells are cells from ATCC strain 43504.

8. A process according to any preceding claim, wherein after exposing the complex to the second antibody, the complex is washed with a buffer that reduces cross-reactivity or otherwise improves the specificity of the assay.

9. A process according to Claim 8, wherein said wash is phosphate buffered saline.

10. A process for the determination of *H. pylori* in a fecal specimen which comprises:

    (a) dispersing a fecal specimen suspected of carrying *H. pylori* in a sample diluent;
    (b) contacting the fecal specimen in the diluent with a first polyclonal antibody for *H. pylori* antigen bound to a solid carrier and a second labelled polyclonal antibody for *H. pylori* to form a complex of the antibodies and the antigen;
    (c) separating said specimen and said complex;
    (d) determining the amount of the labelled antibody and in turn determining the presence of *H. pylori* antigen in said fecal specimen.

11. A process for the determination of *H. pylori* in a fecal specimen which comprises:

    (a) dispersing a fecal specimen suspected of carrying *H. pylori* in a sample diluent;
    (b) contacting the fecal specimen in the diluent with a first polyclonal antibody for *H. pylori* antigen produced by a first antibody-producing species and bound to a solid carrier to form a complex of the antibody and the antigen;
    (c) separating said specimen and said complex;
    (d) contacting the antibody-antigen complex formed in step (b) with a primary polyclonal antibody for *H. pylori* antigen obtained from a sec-

ond antibody-producing species to produce a antibody-antigen-antibody complex:
(e) removing the primary antibody not present in the complex from step (d):
(f) contacting the antibody-antigen-antibody complex formed in step (d) with a secondary antibody, said secondary antibody being an antibody for the second antibody-producing species, whereby said secondary antibody forms a complex with said antibody-antigen-antibody complex; and
(g) determining the presence of *H. pylori* antigen in said fecal specimen.

12. A kit for the determination of *H. pylori* in a fecal specimen including a plate of wells having bound thereto polyclonal antibody for *H. pylori* antigen, a protein-based sample diluent, a conjugate of an enzyme polyclonal antibody for *H. pylori* antigen, wash buffer and, a substrate solution.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number EP 97303147.9

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 6)
A	CHEMICAL ABSTRACTS, vol. 124, no. 13, 1996, March 25, Columbus, Ohio, USA B.A.M. VAN DE WOUW et al. "Comparison of three commercially available enzyme-linked immunosorbent assays and biopsy-dependent diagnosis for detecting Helicobacter pylori infection" page 980, no. 172 810j; & J. Clin. Microbiol. 1996, 34(1), 94-7 (Eng)	1,10, 11	G 01 N 33/569 G 01 N 33/541 G 01 N 33/535 A 61 K 39/106
A	CHEMICAL ABSTRACTS, vol. 120, no. 17, 1994, April 25, Columbus, Ohio, USA A.W. CRIPPS et al. "Rapid in vitro test and kit for Helicobacter pylori in saliva" page 648, no. 215 316g; & AU 644 121	1,10, 11,12	
			TECHNICAL FIELDS SEARCHED (Int. Cl.6)
A	WO - A - 93/22 682 (AUSPHARM INTERNATIONAL LIMITED) * Abstract; claims *	1-3, 10-12	G 01 N 33/00 A 61 K
A,D	US - A - 5 262 156 (M.M. ALEMOHAMMAD) * Totality *	1,10, 11	
A,D	EP - A - 0 329 570 (M.J. BLASER) * Abstract; example 11; claims *	1-3, 5-7, 10,11	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	30-06-1997	SCHNASS

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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